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Virus-Encoded Superantigens

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INTRODUCTION

The term "superantigen" was coined by Kappler and Marrack about 8 years ago to describe the strong T-cell receptor (TCR) Vβ-restricted primary response to bacterial toxins (50). This has led to a fury of work on the functional and structural properties of the new type of immunogen (Fig. 1).

Endogenous murine superantigens, which are inherited in Mendelian fashion, had long been defined functionally (21, 38, 49), but their genes had remained elusive. The discovery, therefore, that they were part of the murine mammary tumor virus (MMTV) proviral DNA which had been integrated into the germ line was very exciting (20, 24, 88). Interest in superantigens further increased with the revelation that the infectious MMTV, a B-type retrovirus which is the causative agent of murine mammary carcinomas, encodes a superantigen (51) and makes use of this molecule for facilitating transmission in the host (27). All superantigens defined so far are of microbial origin; it is possible that these proteins provide an advantage for the respective pathogen by engaging the immune system of the host.

Although tremendous progress in the functional and structural analysis of superantigens has been made in the past few years, the exact composition of the trimolecular complex, consisting of superantigen, major histocompatibility complex (MHC) class II, and TCR Vβ chain, has not yet been resolved. In fact, the model depicted in Fig. 1 may not be entirely correct; we believe that the TCR probably does not contact the MHC class II heterodimer when it interacts with a superantigen but that a bridge is established directly between the superantigen and the outside of the TCR Vβ chain (71). However, this point is controversial among cellular immunologists (90). On the other hand, we do know from the existing cocrystals that the bacterial toxins differ in their interaction with class II, contacting either only the α chain of the MHC protein (36) or both the α and β chains by overlapping the peptide-binding groove (40) (Fig. 2).

Immunological Definitions

MHC, major histocompatibility complex, containing a series of genes that control antigen recognition by T cells. HLA, human MHC; H-2, murine MHC. MHC class I, MHC molecules presenting antigen to cytotoxic T cells ($CD8^+$): HLA.A,

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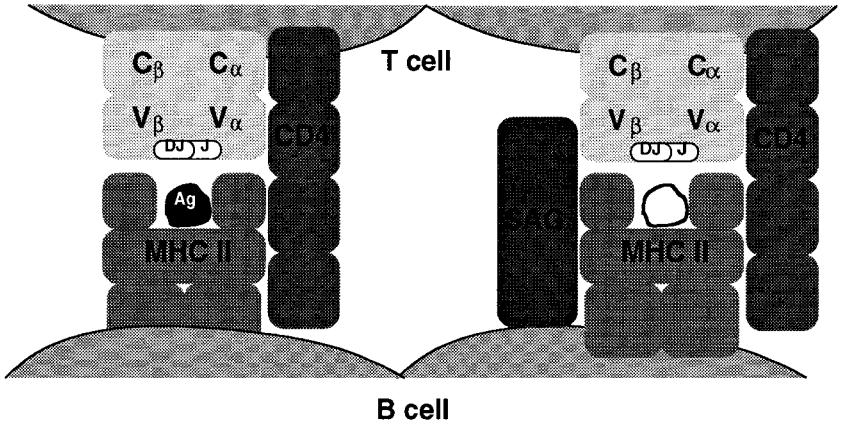

FIG. 1. Model of the trimolecular interaction, involving the TCR, MHC class II, and either a conventional peptide antigen (left) or a superantigen (SAG) (right).

B, and C; H-2K and D. MHC class II, MHC heterodimer presenting antigen to helper T cells ($CD4^+$): HLA.DR, DP, and DQ; H-2 I-A and I-E. DM, heterodimer that controls antigen presentation by class II molecules. Ii, invariant chain associated with the immature form of the class II heterodimer; not encoded within the MHC.

TCR, T-cell receptor, a heterodimer consisting of either an α plus β chain (conventional helper and cytotoxic T cells that express CD4 and CD8, respectively) or a γ plus δ chain (mainly $CD4^-$ $CD8^-$). In this review, we are dealing only with αβ T cells. α chain, V and J (variable region) plus constant region. β chain, V, D, and J (variable region) plus constant region.

Antigen presentation to T cells: (i) conventional peptide antigen—T cells recognize processed antigenic peptides in the context of class I ($CD8^+$ T cell) or class II ($CD4^+$ T cell) glycoproteins; (ii) superantigen—unprocessed antigen that is presented in the context of MHC class II to the TCR Vβ chain.

MMTV, murine mammary tumor virus, a B-type retrovirus. MMTV *sag* is the open reading frame that encodes the superantigen.

Hallmarks of Superantigens

Superantigens differ from conventional peptide antigens in four major aspects. (i) They elicit a strong primary response, while in vivo priming and boosting are necessary to detect T-cell proliferation in vitro in response to normal antigen. It is this feature that has led to the term "superantigen" for the new class of immunogens (50). (ii) The TCR Vβ chain is sufficient for recognition of a superantigen (86), in contrast to that of a conventional peptide antigen, which requires a very specific interaction with the third hypervariable region of the TCR (Fig. 1). Since this region is made up by the joining elements of both VJα and VDJβ, it provides an explanation for the extremely low precursor frequency of T cells responding to a conventional peptide antigen. The murine genome contains only about 15 to 20 functional Vβ genes; therefore, 5 to 10% of T cells are available to mount a response to any superantigen. (iii) All superantigens discovered so far require presentation by MHC class II proteins (23). However, the T-cell response to superantigens is not class II restricted (35, 47), thereby violating the golden rule of MHC restriction that governs all other antigen-specific T-cell responses. A hierarchy exists in the capacity of the various murine class II alleles to present superantigens, $I\text{-}E^k$ being the strongest, followed by $I\text{-}E^d$ and $I\text{-}A^b$, while $I\text{-}A^q$ is virtually unable to present superantigens. Furthermore, we and others have established that superantigens are more efficiently presented by certain human HLA.DR alleles than by mouse MHC class II to both human and murine T cells (33, 71). This observation forms the basis for our model that MHC class II mainly provides a docking structure for the superantigen. Class II alleles contribute different affinities to this interaction, but are not recognized per se by the TCR. (iv) Superantigens are presented in an unprocessed form (14, 23), whereas normal antigens require breakdown into peptides, which are then loaded into the MHC-binding groove (Fig. 1 and 2). The combination of these four features unequivocally defines a superantigen. For example, the tetanus antigen, which elicits a TCR Vβ-restricted response, would not qualify as a superantigen, because priming is essential for detecting this response and the tetanus protein is processed for presentation to the T cells.

Superantigens as Tools for Analyzing T-Cell Selection

Superantigens have served as valuable tools for immunologists to monitor the fate of specifically activated T cells, which can be recognized by anti-Vβ monoclonal antibodies (MAbs). Kappler et al. were the first to prove with endogenous superantigens that clonal deletion is the main mechanism of self-tolerance induction in the thymus (38). Similarly, Bill and Palmer (9) and MacDonald et al. (48) and others could demonstrate that positive selection of thymocytes follows the same rules as negative selection. The important aspect of this work with endogenous superantigens is that no manipulation of the mice was necessary. Furthermore, exogenous superantigens such as bacterial toxins have been used extensively to monitor the fate of activated T cells in the periphery, leading to T-cell tolerance in vivo, either by clonal deletion or by anergy induction (65). Many observations have been reported on the differential T-cell activation capacity of conventional peptide versus superantigen (25, 59, 61, 62), and models have been proposed to explain these results (46). Clearly, the response to a superantigen is controlled by a minimum of two factors: (i) the inherent affinity of a TCR Vβ segment for a particular superantigen, and (ii) the capacity of an MHC class II allele to present the superantigen. Thus, there is a sliding scale in the outcome of the in vivo T-cell reaction to a superantigen, ranging from total clonal deletion to partial anergy induction.

Microbial Origin of Superantigens

All superantigens defined so far are microbial products, implying a role for these molecules in the life cycle of the mi-

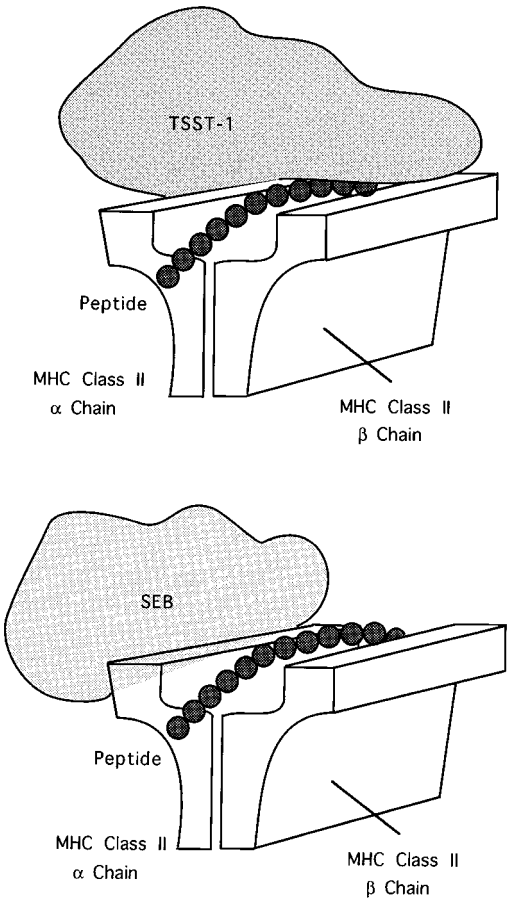

FIG. 2. Differential interaction of bacterial superantigens with MHC class II, according to the published crystal structures (35, 39). TSST-1, toxic shock syndrome toxin 1; SEB, staphylococcal enterotoxin B. Adapted from reference 33a with permission of the publisher.

crobe. The best-studied superantigens are the toxins secreted by certain strains of bacteria. It is intriguing that these superantigens preferentially stimulate the T cells of the host which is infected by the particular bacteria than those of other species (22). This adaptation of the superantigen fine specificity to the TCR Vβ repertoire of the host indicates that the bacteria have learned to make use of the host immune system, most probably for facilitating their own propagation.

The second type of superantigens discovered were encoded by MMTV. In this review, we will discuss the biological significance of these retroviral superantigens in detail, because their role in the transmission of the infectious MMTV is well established. We will also outline the functional description of another superantigen, associated with the herpesvirus Epstein-Barr virus (EBV), and we will consider the possible role that this molecule plays in establishing persistent infection with the double-stranded DNA virus, as well as in fulfilling its oncogenic potential.

MURINE MAMMARY TUMOR VIRUS-ASSOCIATED SUPERANTIGEN

Biological Significance

Facilitation of viral transmission. We have come full circle in our understanding of the relevance of the MMTV superantigen for the completion of the life cycle of infectious MMTV. This B-type retrovirus is transmitted vertically via the milk from mother to offspring (Fig. 3A). From the pioneering work of Ross and her colleagues, we know that superantigen-reactive T cells are required for the migration from the primary residence in the gut of the suckling newborn to the final destination in the mammary tissue (27). It is only here that MMTV replicates spontaneously, as a result of the glucocorticoid-responsive elements in the viral long terminal repeats (LTR), leading, finally, to mammary carcinomas. Replication is vastly enhanced during lactation, and the incidence of mammary tumors increases significantly with each pregnancy. In contrast, viral replication in any other somatic tissue is minimal, and that is why the superantigen-induced stimulation is required. It is well established that a whole cascade of immunological reactions takes place (Fig. 3B), starting with the infected B cell (6, 30, 31), which transcribes the MMTV superantigen and presents it on its cell membrane in the context of MHC class II to superantigen-reactive T cells, expressing the relevant TCR Vβ chain (27, 30, 31). The activated T cells, in turn, stimulate the MMTV-infected B cells by producing growth factors and accessory molecules. Since cellular activation is required for retroviral replication, this stimulation leads to amplification of the virus in the infected B cells, and it is these cells which finally transport the virus to the mammary tissue. It has been shown in great detail by us and others that omission of any of these elements strongly curtails viral transmission: only minimal viral transmission takes place in B-cell-deficient mice (6), and the absence of superantigen-reactive T cells (27, 30, 31) or the lack of MHC class II protein (7) has a similar effect. Thus, this retrovirus has adapted to its host by expressing a molecule that stimulates the immune system cells which are responsible for viral transmission. We will discuss below our evidence that MMTV *sag* may be derived from a primordial eukaryotic gene. Taking MMTV as model system, it is very likely that other pathogens have developed a similar strategy for eliciting an immune stimulation in their host.

Proviral DNA in the germ line as protection against infection. Curiously, the murine genome contains a series of integrated MMTV proviruses as a result of rare integrations of infectious virus into the germ line (13). The majority of these proviruses are fossils with large deletions in their genome which are no longer able to produce infectious viral particles (69). However, they all contain intact *sag* genes encoding superantigens that are expressed in B cells. These determinants, initially known as Mls antigens (21), constitute the family of endogenous superantigens (20, 24, 88). It is likely that an evolutionary force has led to the preservation of these intact *sag* genes in the murine genome, because the endogenous superantigens play a protective role against infectious MMTV (67) (Fig. 3A). The rest of the integrated retrovirus DNA was subject to random deletions and mutations. The endogenous superantigens, which are inherited in Mendelian fashion, cause deletion of reactive T cells as a result of self-tolerance induction in the thymus (37). Thus, the transmission of an infectious virus carrying the identical superantigen will be hampered by the lack of responder T cells (Fig. 3B). This has been elegantly demonstrated by Golovkina et al., who have introduced the *sag* gene of an infectious MMTV as a transgene into the murine genome (27). The resulting mice were highly defective in transmission of this particular infectious MMTV but remained susceptible to an infectious virus with a different *sag* gene.

We have documented a natural example of such a germ line integration event in the MA/MyJ mouse. This strain contains a unique MMTV provirus, not seen in any other inbred or wild mouse, indicating that this particular proviral integration into the germ line happened relatively recently (67). Interestingly,

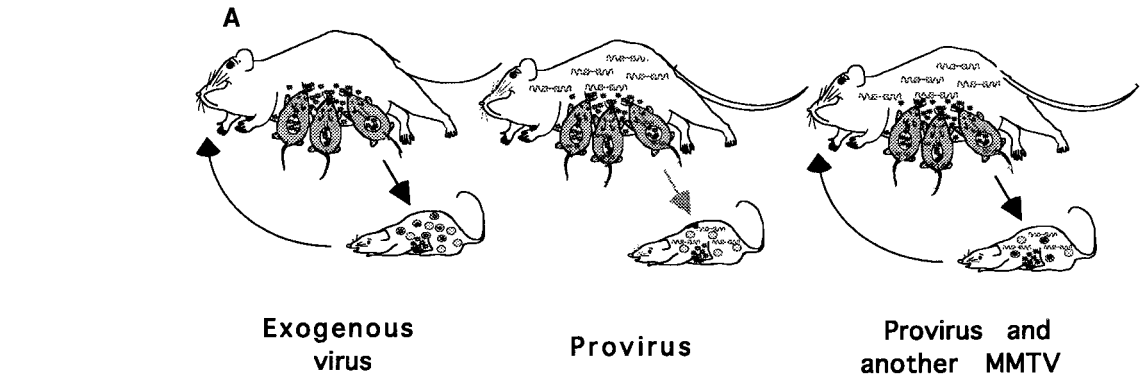

FIG. 3. (A) Transmission of infectious MMTV in the presence or absence of proviral MMTV DNA in the germ line. (B) MMTV in the gut infects B lymphocytes, which leads to superantigen expression. T cells are activated and replicate the virus.

the MA/MyJ strain was derived in the 1940s by Murray from the MA line, which showed a very high incidence of spontaneous mammary carcinoma in virgin and multiparous females (57). In his search for a tumor-resistant animal, Murray noticed one female in a litter of four that did not succumb to mammary carcinomas. Upon breeding this female mouse, he discovered that the female offspring were similarly resistant to oncogenesis. Although not known at that time, the original MA strain must have carried an infectious MMTV, and germ line integration occurred in a predecessor of this one female mouse which was resistant to tumorigenesis. Thus, this mouse did not contain T cells reactive to the superantigen of the infectious virus, rendering it resistant to the carcinogenic potential of the virus.

We hypothesize that the germ line-integrated MMTV *sag* genes have been maintained intact in the genome, because they provide protection against infection with this highly oncogenic virus (67). Our model is supported by the fact that virtually all inbred and wild mice analyzed to date carry several integrated fossil MMTV proviruses which have intact polymorphic *sag* genes (1). On the other hand, this murine strategy of defense has led to the development of polymorphism in the MMTV superantigen, because the only way a virus could be amplified in an animal containing a germ line-integrated proviral *sag* gene would be if it had a mutated *sag* gene with a different TCR Vβ specificity. Since the retroviral evolution is much faster than the eukaryotic one, infectious oncogenic MMTV has survived through the acquisition of polymorphism in its *sag* gene. Interestingly, the MMTV genome of the various strains is highly conserved, except for the polymorphism seen in the U3 region of the 3′ LTR, where the *sag* gene is encoded (5) (Fig. 4).

Structural Features

MMTV, a B-type retrovirus, contains the same structural genes as any C-type retrovirus, such as murine leukemia virus, with one exception: MMTV has an open reading frame in the U3 region of the 3′ LTR (17) (Fig. 4). Although this unique feature was noticed by virologists a long time ago (18), the discovery that it encodes a superantigen was made only recently (2, 5, 11, 63, 89). The open reading frame has therefore been renamed *sag* to indicate its function. The protein encoded by this *sag* gene is a type II transmembrane molecule of about 45 kDa, with the C terminus outside the cell membrane and the N terminus in the cytoplasm (5, 12, 41, 42). The polymorphic region is restricted to the last 10 to 14 amino acids at the C terminus, encoding the TCR Vβ specificity, while the rest of the gene is highly conserved in the various MMTV strains. Two arginine-rich motifs, RARR and RKRR, are found in the extracellular region of the superantigen, which are likely cleavage sites for a furin-type protease, as implicated from mutational analyses (60).

Association with MHC class II. From all the functional data, we conclude that the MMTV superantigen is presented in the context of the MHC class II heterodimer, similar to the bacterial superantigens, which are secreted molecules. We have confirmed the requirement for this association by the use of MHC class II-deficient mice, which cannot present superantigens and thus are unable to transmit infectious MMTV, nor can their spleen cells stimulate an Mls-specific T-cell proliferation in vitro (7).

How does an endogenously synthesized viral protein associate in unprocessed form with class II? The textbook descrip-

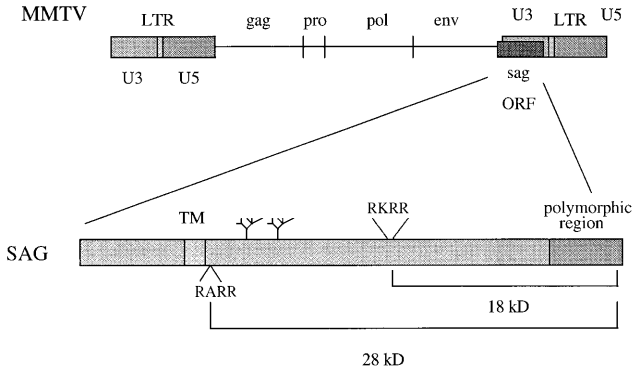

FIG. 4. MMTV retrovirus and protein structure of the *sag* gene product.

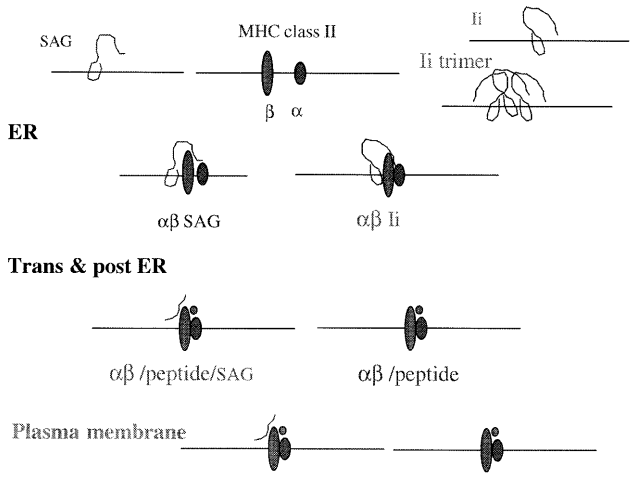

FIG. 5. Model of MHC class II association with Ii or superantigen (SAG) in the ER.

tion is that an infected cell presents viral proteins mainly in the context of MHC class I, after breakdown of the newly synthesized molecules in the cytoplasm by the proteasome complex (19) and transport of the resulting peptides into the endoplasmic reticulum (ER), with the help of the transporter associated with antigen processing (72). Class II, on the other hand, is specialized in presenting exogenous antigen that is taken up by phagocytosis and processed into peptides in the endosomes. Recently, it has been discovered that the process of peptide uptake in the endosomes is catalyzed by the DM gene products (15, 70). By transfecting the *Mtv-7 sag* gene into DM mutant cell lines, we have observed unimpaired superantigen expression, indicating that the MMTV superantigen associates with class II independently of the DM heterodimer (34). This is consistent with the notion that the functional MMTV *sag* product is not processed into a peptide, similar to the bacterial toxins (14, 23). The only protein known to associate in unprocessed form with the class II heterodimer in the ER is the invariant chain, Ii (Fig. 5).

Ever since we first cloned the *Mtv-7 sag* gene, we noticed the overall similarity of its structure to that of Ii: both are type II transmembrane glycoproteins of 35 to 40 kDa (Fig. 6), and both are known to associate with class II. It is well established that the Ii makes use of the CLIP motif to bind to class II; from the recently published crystal structure, we have learned that CLIP binds to the class II cleft like a conventional peptide (26). We have scanned the MMTV superantigen amino acid sequence for a motif that fulfills the requirements for class II binding, with help of the formula developed by Hammer et al. (28). Using the P1 position of class II-associated peptides as a screen, we have identified one particular peptide which fulfills all requirements and has a high class II-binding score. Interestingly, this motif lies in a conserved region of the extracellular domain of the MMTV superantigen, at a similar position to the CLIP in the Ii chain. The two class II-binding motifs are about the same distance from the transmembrane segments, but they do not show direct amino acid homology (Fig. 6) (34). We have used the following two approaches to document binding of MMTV superantigen to class II.

(i) Analysis of the mature proteins. Using papain-cleaved, soluble HLA.DR1 class II molecules and the extracellular part of recombinant *Mtv-7 sag* (Fig. 4), we were able to demonstrate specific binding to class II in Western blots (immunoblots), as well as in BIAcore biosensor analyses (56). Papain-cleaved soluble HLA.A2 class I protein served as negative control in these studies, and no binding of the MMTV superantigen fragments to this MHC molecule was observed. Equal binding was demonstrated with the whole extracellular *Mtv-7* superantigen domain of 28 kDa, as with the 18-kDa C-terminal fragment, corresponding to the second furin cleavage site (Fig. 4). These data correlate well with the biochemical analyses of Winslow et al., who observed an 18-kDa MMTV fragment, containing the C-terminal polymorphic peptide, on the cell membrane (87).

(ii) Association during biosynthesis. While the above analyses yielded significant results, they did not reveal how the association of the superantigen with class II takes place during biosynthesis. For this purpose, we used an in vitro cotranslation system, developed by Bijlmakers et al. (8), to establish the biosynthetic association of the Ii chain with class II, depending on the CLIP motif (8). Our data provide evidence that the MMTV superantigen may associate with the class II heterodimer during biosynthesis through the putative CLIP peptide, similar to the Ii chain (34).

Eukaryotic Origin of the MMTV Superantigen

From the various analyses of the MMTV *sag* gene product, we can draw the following conclusions: (i) MHC class II is essential for presentation of this superantigen in vitro as well as in vivo; (ii) conventional antigen processing is not required, because presentation is independent of the DM heterodimer which promotes Ii CLIP-peptide exchange in the endosomes; and (iii) the superantigen protein structure has a striking sim-

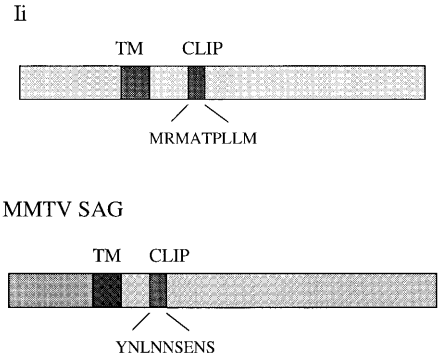

FIG. 6. Comparison of protein structures of Ii and superantigen (SAG), with respective CLIP peptides.

ilarity to that of the class II-associated Ii chain, extending to a CLIP-like motif in the superantigen. Therefore, we suggest that the infectious MMTV has trapped a cellular gene during passage in its host and has maintained it because it provides a selective advantage for transmission. This eukaryotic DNA seems to have contained a primordial gene that gave rise to the Ii chain, on the basis of the structural similarities between the *sag* and the Ii gene products. It is well documented that viruses have the capacity to incorporate host genes into their genome and that such genes are maintained only if they provide a selective advantage for the infectious organism.

RABIES VIRUS-ASSOCIATED SUPERANTIGEN

The nucleocapsid protein of rabies virus has recently been reported to be a superantigen in humans, stimulating Vβ8 T cells (43). Similarly, rabies virus adapted for infection of mice expresses a superantigen which specifically stimulates murine T cells in a Vβ-specific manner (44, 45). While this negative-strand RNA virus does not require cellular activation for replication, it seems, nevertheless, that the superantigen-induced stimulation of T cells facilitates viral transmission from the initial site of infection (the muscle tissue) to the nerve endings. This has been elegantly demonstrated in the murine system, in which deletion of superantigen-reactive T cells delayed the transmission of the rabies virus to the nerve endings (44). Furthermore, a protective role of immunization with the nucleocapsid protein has been demonstrated in the mouse (44, 45). Thus, although we do not yet fully understand the underlying cellular mechanism by which the superantigen-induced T-cell stimulation promotes rabies virus transmission, the biological effects are evident. It is likely that a cytokine secreted by the superantigen-stimulated T cells facilitates viral transmission.

EPSTEIN-BARR VIRUS-ASSOCIATED SUPERANTIGEN

EBV is a human herpesvirus that contains a genome of 172 kb of double-stranded DNA and encodes over 100 genes (4). Its tropism is for primary B lymphocytes (39, 52). The EBV genome is linear in the virus particle but exists as a circular episome inside the latently infected cell. EBV binds to CR2 (CD21) on B cells and is internalized by receptor-mediated endocytosis. Two types of cellular infection can occur: (i) lytic infection, leading to viral replication and lysis of the host cell, and (ii) latent infection, leading to chronic infection of the host cell.

Clinical Observations

Infectious mononucleosis. EBV is transmitted via saliva and infects nasopharyngeal epithelial cells and B lymphocytes; it is endemic in all adult populations. Most people are exposed to EBV in early childhood and carry the virus for life with no apparent consequences. However, if EBV infection is postponed to adolescence, about 50% of the affected individuals develop infectious mononucleosis (IM) (32), a self-limiting lymphoproliferative disease (64). $CD8^+$ cells are mainly responsible for the lymphocytosis at the height of IM (79), but $CD4^+$ T cells are activated initially (64, 78). The self-limiting nature of the elicited T-cell proliferation provided the rationale for us to look for the action of a superantigen (75).

Oncogenic effects in immunosuppressed individuals. Although EBV is endemic in adults, its oncogenic effects are only seen in certain ethnic groups or in immunosuppressed individuals. In Africa, EBV is the causative agent of Burkitt's lymphoma, which is prevalent mainly in areas where malaria is widespread. It is thought that the general immunosuppressed state inflicted by malaria contributes to the development of EBV lymphomas. In the Chinese population, EBV causes nasopharyngeal carcinoma. Little is known about the etiology of this disease.

On the other hand, it is well established that immunosuppressed individuals, most notably organ or bone marrow transplant recipients and AIDS patients, frequently develop EBV B-cell lymphomas. Recently, the SCID/hu mouse has become a model for EBV-associated B-cell proliferative disorders. It has been demonstrated that EBV B-cell lymphomas arise in SCID mice reconstituted with peripheral blood mononuclear cells (PBMC) of EBV^+ individuals only if T cells are cotransferred. This seems paradoxical, since the cytotoxic T cells control the spread of EBV-infected virus-producing cells in normal individuals (78). We will discuss this topic below in more detail.

Experimental Observations: In Vitro Model System

To analyze the initial events that lead to the strong T-cell proliferation during IM, we set up an in vitro assay system. This was necessary because the clinical diagnosis of IM is reached only well into the disease, when T-cell activation is at its height. Therefore, we transformed B lymphocytes in vitro with various EBV isolates (58) and then tested the stimulatory capacity of the resulting lymphoblastoid cell lines (LCL) on autologous T cells, isolated from the PBMC (75).

Strong T-cell proliferative response. Setting up the above culture system, we observed vigorous T-cell proliferation that peaked early, similar to a mitogen response. Since virtually all adults are EBV^+ by serology, we could not exclude the possibility of a recall antigen response. However, the magnitude and the early kinetics of the proliferation seemed to rule out a conventional memory response. To unequivocally settle this question, we tested cord blood lymphocytes, which, by definition, are 100% fetally derived and hence EBV^-, because herpesviruses are not transmitted to the fetus during pregnancy. We observed strong proliferation of the fetal T cells, with an early peak of activation, as seen in PBMC of adult donors.

TCR Vβ-restricted response. The hallmark of a superantigen response is the TCR Vβ specificity of the response. Because of the magnitude of the elicited response, it was necessary for us to design an assay that detects T-cell activation before proliferation and lymphokine production take place. This prevented the readout of bystander T cells that are usually activated in a strong response and thus obscure the initial TCR Vβ specificity. We achieved this by determining the acquisition of an early activation marker, CD69 (29), on the various T-cell Vβ subsets 4 h after in vitro stimulation. Using this readout, we could demonstrate that Vβ13 T cells were preferentially activated, regardless of the origin of the EBV used for deriving the LCL. This assay has the advantage that it can measure only a polyclonal response, ruling out a massive expansion of a clonal recall antigen response. The use of murine T-cell hybridomas that express selective human TCR Vβ genes allowed us to confirm these results; namely, only $Vβ13^+$ cells were activated, and the various family members showed differential sensitivity to EBV-induced stimulation (75).

MHC class II-dependent presentation. To classify the EBV-stimulatory activity as a superantigen, it was necessary to demonstrate the class II dependence of the EBV LCL-induced T-cell stimulation. This was achieved with anti-class II blocking antibodies, which were added to the cultures at the onset of stimulation. While the mitogen response was not influenced by this addition, the EBV LCL-induced stimulation was com-

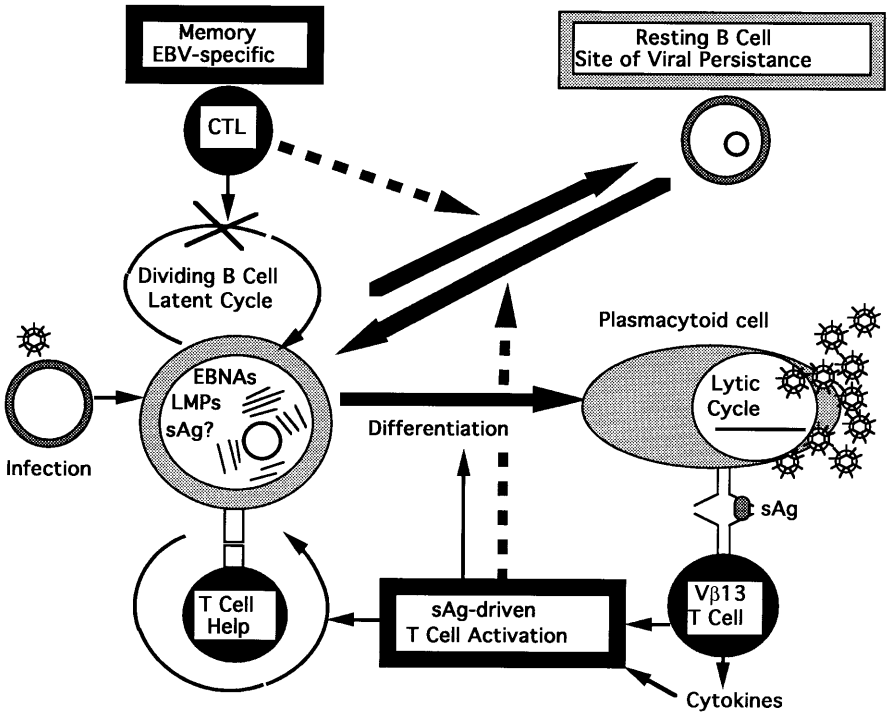

FIG. 7. Model of the biological significance of EBV-associated superantigen (sAg). The role of the superantigen in persistent infection is to provide T-cell help to latently infected B cells, supplying the signals necessary for initiation of growth and differentiation. This results in periodic viral replication. EBV-specific cytotoxic T lymphocytes (CTL) work in an antagonistic manner, killing actively dividing, latently infected B cells and thus selecting for a population of cells that downregulates EBV gene expression. This population becomes the site of viral persistence. During immunosuppression, the memory cytotoxic T-lymphocyte response is compromised whereas superantigen stimulation of primary T cells is unaffected. As a consequence, unregulated B-cell growth can result in tumor formation. LMP, latent membrane protein.

pletely blocked. Thus, we believe that we have functionally demonstrated the EBV-associated activity of a superantigen (75).

Model of Biological Significance

An EBV-encoded superantigen should provide a selective advantage for this herpesvirus, presumably for substantiating infection. This is the main purpose of the virus, while the oncogenic potential may be a side effect that has no consequences for viral spread. We will discuss the biological significance of an EBV-associated superantigen from the viewpoint of the virus (Fig. 7).

Facilitation of viral transmission. It is possible that superantigen-induced T-cell stimulation is required for establishing persistent infection in B cells. Although it is well known that the EBV latent genes directly induce B-cell proliferation in vitro, it has recently been shown that persistent infection in vivo resides in resting B cells, which do not express the growth-promoting latent genes (53). It is likely, therefore, that superantigen-activated T cells provide the necessary survival signals for these latently infected B cells, allowing them to grow in the absence of a viral signal. The CD40/CD40L interaction between activated T and B cells seems ideally suited for this purpose. Furthermore, T-cell signals may be required for inducing latently infected resting B cells to express the growth-promoting latent genes and consequently to divide. In this manner, B cells replicate the viral genome without actually producing infectious virus. Moreover, T-cell signaling might push the B cells to terminally differentiate and undergo lytic replication of EBV, thus completing the life cycle of the virus (Fig. 7).

Oncogenic potential in immunosuppressed individuals. It had been observed previously that spontaneous outgrowth of LCL in vitro from EBV^+ PBMC requires the presence of T cells (85). This T-cell dependence is obscured by the later development of EBV-specific cytotoxic T lymphocytes, which eliminate the newly generated LCL (55, 77). Our model fits data obtained in vivo in SCID mice transplanted with EBV-seropositive human PBMC (82–84). These mice spontaneously developed EBV-associated B-cell lymphomas only when T cells were cotransferred, suggesting that a direct T-cell–B-cell interaction is required. Furthermore, it has recently been demonstrated that the tumors arising in SCID mice are a mixture of terminally differentiated B cells, which replicate the virus, and proliferating B cells, which are latently infected (66). It is likely that the nondividing differentiated cells provide an essential growth component for the tumor, namely, superantigen-induced activation of T cells. These T cells would push the latently infected B cells to continuously proliferate while simultaneously allowing some fraction to terminally differentiate, leading to growth arrest and production of virus, as well as to more superantigen. Thus, tumor growth may represent a type of equilibrium between proliferation and differentiation. Long-term growth selection, however, would lead to outgrowth of cells that have mutated away from the requirement for the superantigen, resulting in tumors no longer containing differentiated cells. This is precisely the characteristic of tumors that arise in SCID mice after long periods (66). We propose, therefore, that the SCID/hu mouse model supports an essential role of the viral superantigen in EBV-induced oncogenesis.

An analogous murine disease model has been described in detail for the development of reticular cell sarcoma in SJL

mice (80, 81). This B-cell lymphoma expresses elevated levels of an endogenous MMTV superantigen, causing T-cell stimulation, which, in turn, drives the proliferation of the newly transformed B cells, promoting tumor formation. This phenomenon has been termed reverse immunosurveillance, because the T cells encourage tumor growth instead of eliminating the cancerous cells. The identification of an EBV superantigen could therefore be important from a clinical standpoint, because it provides a rationale for specific immunotherapy, preventing EBV lymphomas in immunosuppressed patients; i.e., the superantigen-induced activation of Vβ13 T cells could be eliminated.

Autoimmunity. EBV has been linked to the induction of autoimmunity, especially in Sjögren's syndrome, which is associated with elevated levels of this herpesvirus in patients (68). Of note are reports describing a significant increase in the number of Vβ13 T cells in the lesions of patients with this autoimmune disease, indicative of the possible action of an EBV-associated superantigen (73, 74, 91).

CYTOMEGALOVIRUS-ASSOCIATED SUPERANTIGEN

Superantigen activity has recently been attributed to another herpesvirus, cytomegalovirus (CMV), causing activation of Vβ12 T cells (16). This virus is also ubiquitous in the general population. It infects monocytes, which are antigen-presenting cells, like the B cells, the targets of EBV. Interestingly, exposure to CMV during adolescence leads to IM-like symptoms, characterized by massive T-cell proliferation which is self-limiting, whereas infection during childhood is usually symptom free. Thus, these two herpesviruses elicit similar immunological reactions in the host.

The only readout of superantigen activity of CMV so far comes from studies of AIDS patients. It was discovered that human immunodeficiency virus type 1 (HIV-1) replication is enhanced in Vβ12 T cells in HIV-1-infected CMV^+ patients but not in HIV-1-infected CMV^- newborns (16). This phenomenon could be replicated in vitro by the demonstration that HIV-1 replication is specifically enhanced in Vβ12 T cells in the presence of a CMV-infected cell line. The authors attribute their in vivo finding to the reactivation of CMV during HIV-1 infection. The T-cell stimulation is very moderate, since no expansion of the Vβ12 T cells is seen. However, it is very effective, because the activated T cells seem to serve as a reservoir for HIV-1 over a period of years in infected patients.

It is tantalizing to speculate that EBV plays a similar role in HIV-1 infection, because it is even more prevalent than CMV in the general population (76). Other herpesviruses may have similar functions. Recently, a novel herpesvirus that is believed to contribute to the development of Kaposi's sarcoma (54) and, possibly in conjunction with EBV, to the development of body cavity B-cell lymphomas (10) has been identified. This cryptic herpesvirus is thought to reside mainly in antigen-presenting B cells that infiltrate the Kaposi's sarcoma lesion (3). Thus, it is possible that this novel herpesvirus has also evolved a means of exploiting the host immune system.

CONCLUSION AND FUTURE DIRECTIONS

Although tremendous progress in the functional and structural characterization of microbial superantigens has been made in the past few years, we believe that we have only just begun our quest. It is very likely that apart from the few species discovered so far, other microbes also have acquired a superantigen, because stimulation of the host immune system is advantageous for their propagation. Thus, we predict that significant new insights into the pathogenesis of microbial diseases will be gained in the future by the analysis of this new type of antigen.

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